PB85-207470

Morphometric Studies of the Effects of Ozone on Rodent Lungs

Duke Univ. Medical Center, Durham, NC

Prepared for

Health Effects Research Lab.
Research Triangle Park, NC

May 85

U.S. Department of Commerce
National Technical Information Service
NTIS

PB85-207470

EPA/600/1-85/008
May 1985

MORPHOMETRIC STUDIES OF THE EFFECTS OF OZONE ON RODENT LUNGS

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EPA COOPERATIVE AGREEMENT CR807255020

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This study was conducted in cooperation with
Duke University Medical Center

Health Effects Research Laboratory
Office of Research and Development
U. S. Environmental Protection Agency
Research Triangle Park, N. C. 27711

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TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO.	2.	3. RECIPIENT'S ACCESSION NO.
EPA/600/1-85/008		PB85 207470/AS

4. TITLE AND SUBTITLE	5. REPORT DATE
MORPHOMETRIC STUDIES OF THE EFFECTS OF OZONE ON RODENT LUNGS	May 1985
	6. PERFORMING ORGANIZATION CODE

7. AUTHOR(S)	8. PERFORMING ORGANIZATION REPORT NO.
B.E. Barry, J.D. Crapo	

9. PERFORMING ORGANIZATION NAME AND ADDRESS	10. PROGRAM ELEMENT NO.
Departments of Medicine and Pathology Duke University Medical Center Durham, North Carolina 27710	11. CONTRACT/GRANT NO. CR-807255020

12. SPONSORING AGENCY NAME AND ADDRESS	13. TYPE OF REPORT AND PERIOD COVERED
Office of Research & Development Health Effects Research Laboratory USEPA Research Triangle Park, NC 27711	Final
	14. SPONSORING AGENCY CODE EPA-600/11

15. SUPPLEMENTARY NOTES

P.O. Jean Wiester

16. ABSTRACT

The degree of lung injury caused by prolonged inhalation of low levels of ozone is relevant because a number of urban environments periodically reach levels of 0.2 - 0.3 ppm ozone. Morphometric methods were used to evaluate the effects of 0.25 ppm ozone on lung tissue of both young adult and juvenile rats. Three regions of the lung were examined, the distal alveolar region, the proximal alveolar region, and the terminal bronchioles. In addition, the effects of inhalation of 0.12 ppm O_3 on the proximal alveolar region of young adult rats were also evaluated.

The results showed that ozone in these low concentrations reacts mainly in the terminal bronchioles and the proximal alveolar region.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS *(This Report)*	21. NO. OF PAGES
		39
	20. SECURITY CLASS *(This page)*	22. PRICE

EPA Form 2220-1 (Rev. 4-77) PREVIOUS EDITION IS OBSOLETE

NJTICE

Although the information in this document has been funded wholly or in part by the United States Environmental Protection Agency under cooperative agreement No. CR807255020, it does not necessarily reflect the views of the Agency and no official endorsement should be inferred.

ABSTRACT

The degree of lung injury caused by prolonged inhalation of low levels of ozone is relevant because a number of urban environments periodically reach levels of 0.2-0.3 ppm ozone. Morphometric methods were used to evaluate the effects of 0.25 ppm ozone on lung tissue of both young adult and juvenile rats. Three regions of the lung were examined, the distal alveolar region, the proximal alveolar region, and the terminal bronchioles. In addition, the effects of inhalation of 0.12 ppm O_3 on the proximal alveolar region of young adult rats were also evaluated.

Samples of distal alveolar regions were randomly selected from the left lobe of each animal. Samples of the proximal alveolar region and terminal bronchioles were isolated by microdisection. All samples were prepared for electron microscopy and EM morphometric analysis.

The results showed that ozone in these low concentrations reacts mainly in the terminal bronchioles and the proximal alveolar region. After 6 weeks of inhaling 0.25 ppm ozone, the arithmetic mean thickness of the epithelium and endothelium in the distal alveolar region increased 10% ($p=0.08$) and 16% ($p=0.08$) respectively, in mature rats. No other significant changes were found in the morphometric characteristics of the distal alveolar region of the adult or neonatal rats. In the proximal alveolar region, where the effect of ozone was most evident, the number of type I epithelial cells doubled in the animals exposed from 1 day of age. The mean surface area of type I epithelial cells decreased 38% and their mean thickness increased 24%. The number of alveolar type II epithelial cells also increased and the number of alveolar macrophages doubled. Young adult animals exposed to 0.25 ppm ozone showed similar changes in the epithelium of the proximal alveolar region, moreover, these animals showed a doubling of interstitial macrophages indicating a mild inflammatory stimulus in the interstitium. In the terminal bronchioles, exposure to 0.25 ppm ozone produced a significant 14-16% decrease ($p<0.05$) in the average luminal surface area of clara cells. Inhalation of 0.12 ppm ozone for 6 weeks also caused measurable changes in the proximal alveolar region of young adult rats. Type I cell number increased 36% ($p<0.05$) and the mean surface area of both the air side and the basement membrane side of the cell decreased 23%. These results suggest that 0.25 ppm ozone causes epithelial injury in the proximal alveolar region and in the terminal bronchioles of both juvenile and young adult rats. Exposure to 0.12 ppm ozone causes a detectable but less extensive injury to the type I epithelium in the proximal alveolar region.

CONTENTS

Abstract.....iii

List of Tables.....v

List of Figures.....vi

Acknowledgements.....vii

1. Introduction.....1
2. Conclusions and Recommendations.....2
3. Materials and Methods.....3
   - Test Strain.....3
   - Exposures.....3
   - Fixation of lung tissue.....4
4. Experimental Procedures.....5
   - Morphometric study of the distal alveolar region.....5
   - Morphometric study of the proximal alveolar region.....5
   - Morphometric study of the terminal bronchiole.....6
5. Results.....9
6. Discussion.....14

References.....17

Tables.....21

Appendices

A. List of Publications

B. Barry BE, FJ Miller and JD Crapo. Alveolar epithelial injury caused by inhalation of 0.25 ppm of ozone. Adv Mod Environ Toxicol 3:299-309, 1982.

C. Crapo JD, BE Barry, L Chang and RR Mercer. Alteration in lung structure caused by inhalation of oxidants. J Toxicol and Environ Health, In press, 1984.

D. Barry BE and JD Crapo. Application of morphometric methods to study diffuse and focal injury in the lung caused by toxic agents. CRC Critical Review in Toxicology, In press, 1984.

E. Barry BE, FJ Miller and JD Crapo. Effects of inhalation of 0.25 ppm ozone on the proximal alveolar region of juvenile and young adult rats. Submitted, Laboratory Investigation, 1984.

LIST OF TABLES

Table 1 Absolute volumes, surface areas and diffusion capacity for parenchymal portion of the lungs of 12 week old rats exposed to 0.25 ppm ozone since 6 weeks of age (distal alveolar region).....21

Table 2 Morphometrically determined characteristics of the major parenchymal lung cells in 12 week old rats exposed to 0.25 ppm ozone since 6 weeks of age (distal alveolar region).....22

Table 3 Absolute volumes, surface areas and diffusion capacity for parenchymal portion of the lungs of 1 week old rats exposed to 0.25 ppm ozone since 1 day of age (distal alveolar region).....23

Table 4 Morphometrically determined characteristics of the major parenchymal lung cells in 1 week old rats exposed to 0.25 ppm ozone since 1 day of age (distal alveolar region).....24

Table 5 Absolute volumes, surface areas and diffusion capacity for parenchymal portion of the lungs of 6 week old rats exposed to 0.25 ppm ozone since 1 day of age (distal alveolar region).....25

Table 6 Morphometrically determined characteristics of the major parenchymal lung cells in 6 week old rats exposed to 0.25 ppm ozone since 1 day of age (distal alveolar region).....26

Table 7 Absolute volumes, surface areas and diffusion capacity for parenchymal portion of the lungs of 12 week old rats exposed to 0.12 ppm ozone since 6 weeks of age (proximal alveolar region).....27

Table 8 Morphometrically determined characteristics of the major parenchymal lung cells in 12 week old rats exposed to 0.12 ppm ozone since 6 weeks of age (proximal alveolar region).....28

Table 9 Morphometrically determined characteristics of terminal bronchiolar cells in the lungs of 6 week old rats exposed to 0.25 ppm ozone since 1 day of age.....29

Table 10 Morphometrically determined characteristics of terminal bronchiolar cells in the lungs of 12 week old rats exposed to 0.25 ppm ozone since 6 weeks of age.....30

List of Figures

Figure 1 Effects of inhalation of 0.25 ppm ozone on the characteristics of type I epithelial cells in the proximal alveolar region of juvenile and young adult rats.....10

Figure 2 Effects of inhalation of 0.25 ppm ozone on the characteristics of type II epithelial cells in the proximal alveolar region of juvenile and young adult rats.....10

Figure 3 Effects of inhalation of 0.12 ppm or 0.25 ppm ozone on the total tissue volumes of the various tissue compartments in the proximal alveolar region of young adult rats.....11

Figure 4 Effects of inhalation of 0.25 ppm ozone on the characteristics of ciliated cells in the terminal bronchioles of juvenile and young adult rats.....12

ACKNOWLEDGEMENTS

Dr. Judy Graham and Dr. Fred Miller, U.S. Environmental Protection Agency, provided essential help in the design of these experiments and in supervision of the animal exposures.

The animal exposures were carried out by personnel of Northrop Corporation under contract to the U.S. Environmental Protection Agency.

Southern California Edison Company provided partial support for a portion of these studies.

INTRODUCTION

Ozone is an atmospheric pollutant occurring as a photochemical oxidant in ambient air. It is a product of an atmospheric photochemical reaction between organic and inorganic pollutants and atmospheric oxygen. The actual mechanism of ozone toxicity at the subcellular level is not known. Possible explanations of toxicity include: 1) oxidation of polyunsaturated lipids in cell membranes; 2) oxidation of sulfhydryl groups; 3) formation of toxic compounds (ozonides) through reactions with polyunsaturated lipids; 4) formation of free radicals; 5) nonspecific stress with release of histamine; and, 6) injury mediated by pharmacologic action, such as via a neurohormonal mechanism (EPA, 1978; Menzel, 1970).

The concentration of ozone in ambient air depends on the concentration of the primary pollutants as well as local environmental conditions such as temperature, air stagnation and sunlight intensity. For several years oxidant concentrations have been measured by state and local control agency monitoring stations. These data are gathered and analyzed by the Environmental Protection Agency. Most urban centers have intermittently exceeded the current national air quality standard for ozone (0.12 ppm), and several cities (Los Angeles, Denver, Philadelphia, Houston and New York) have had high hourly levels in excess of 0.20 to 0.30 ppm. The chronic effects of such ozone concentrations are unknown.

Currently there is a scientific agreement that ozone concentrations in excess of one ppm are hazardous to human health. Experiments on laboratory animals (rats, mice, guinea pigs, rabbits and monkeys) at ozone concentrations of 0.2 to 1.0 ppm for short exposures have demonstrated qualitative histopathologic changes in conducting airways and alveolar spaces. Various investigations have described: 1) damage to ciliated cells in the terminal bronchioles with nonciliated progenitor cell division for bronchiolar epithelial cell renewal (Evans et al. 1976; Schwartz et al., 1976); 2) hyperplasia and hypertrophy of nonciliated bronchiolar epithelial cells in respiratory bronchioles and intrabronchial accumulation of alveolar macrophages (Mellick et al., 1977); 3) significant cellular damage at the alveolar duct level (Dungworth, 1976); 4) swelling and desquamation of type I alveolar lining cells with subsequent proliferation of type II cells to cover denuded areas (Schwartz et al., 1976; Boatman et al., 1974; Stephens et al., 1974); and, 5) ultrastructural evidence of damage to endothelial cells and the basement membrane (Bils, 1970). However, there are currently no qualitative or quantitative morphologic studies of animals exposed to ozone concentrations less than 0.2 ppm. Such experiments are obviously vital to our understanding of possible damage to the human lung in urban areas, where low level exposures occur intermittently in the range of 0.20 ppm or less.

CONCLUSIONS AND RECOMMENDATIONS

EM morphometry is extremely sensitive and particularly suited as a tool to evaluate health effects of air pollutants such as ozone at low concentrations. By the combined use of microdisection and EM morphomeetry, we demonstrated in this study that subchronic exposure to 0.25 ppm ozone produced significant structural changes in the cells of the terminal bronchioles and their adjacent alveolar tissues. In addition, inhalation of 0.12 ppm O_3, the current ambiant air quality standard, caused structural changes to occur in the alveolar epithelium immediately peripheral to the terminal bronchioles.

A wide array of effects have previously been reported as a result of subchronic exposure to ozone. These investigations, although mostly at higher concentrations of O_3, were essentially in agreement with the results of the present study in the location and nature of the injury invoked by ozone. The major impact of the current study is that by using quantitative techniques subchronic exposure to ozone at extremely low levels can be shown to cause structural changes in lung cells which are suggestive of injury. These changes can be detected at the current air quality standard of 0.12 ppm O_3. Because the long term effects of lung cell injury caused by low levels of O_3 are not known, chronic exposure to O_3 at near ambient levels should be performed and adverse health effects carefully defined.

MATERIALS AND METHODS

Test Strain

Specific pathogen free male, Fischer 344 rats [CDF (F-344)]/Cr 1BR] that were either 1 day or 6 weeks old at the initiation of the exposures were used. In order to avoid the chronic murine pneumonia which is endemic in laboratory rats, it was essential that the animals be specific pathogen free. The exposures were carried out under as clean an environment as possible. All animals were supplied by the Environmental Protection Agency.

Exposures

Six week old male Fischer 344 rats were placed in stainless steel chambers for exposure to air or ozone. Ozone was generated from 100% oxygen with an Orec Model 03V5-0 ozonizer (Ozone Research and Equipment Corp., Phoenix, Arizona) mixed with dry filtered air and directed into the top of the chamber. Rats were continuously exposed to filtered air and 0.12, or 0.25 ppm of ozone for 12 hours per day, 7 days per week for 6 weeks. At the end of the exposure lungs were fixed and total lung volumes determined.

A. Mature Rats - All animals were 6 weeks old at the initiation of the following exposures. All exposures have been done by the EPA.

# Animals	Type of Exposure	Duration of Exposure	
8	.12 ppm Ozone	1 week	12 hrs/day
8	"	6 weeks	"
8	.25 ppm Ozone	1 week	12 hrs/day
8	"	6 weeks	"
12	Air Controls	1 week	
12	"	6 weeks	

All exposure groups were weight matched at the beginning of the experiment.

B. Neonatal Rats - All animals were born in the exposure facility and exposures were initiated 24 hours after birth. The pups were weaned at 3 weeks of exposure.

# Animals	Type of Exposure	Duration of Exposure	
8	.12 ppm Ozone	1 week	12 hrs/day
8	"	6 weeks	"
8	.25 ppm Ozone	1 week	12 hrs/day
8	"	6 weeks	"

B. Neonatal Rats (continued)

# Animals	Type of Exposure	Duration of Exposure
12	Air Controls	1 week
12	"	6 weeks

All exposure groups were age matched and all litters were matched in size with male pups only at the beginning of the experiment.

Fixation of lung tissue

For histologic and morphometric studies, rats were anesthetized with intraperitoneal pentobarbital; in rapid succession, the abdomen was opened, both hemidiaphragms were punctured to allow collapse of the lungs, the trachea was cannulated, and fixative was infused down the trachea at a constant pressure of 20 cm of fixative above the midpint of the chest wall. The fixative was 2% glutaraldehyde in 350 milliosmolar cacodylate buffer at pH 7.4 (Weibel, 1970). After fixation the lung volume was determined by volume displacement (Burri et al., 1974; Scherle 1970).

EXPERIMENTAL PROCEDURE

In order to quantitate changes occuring in the lungs of these animals morphometric techniques were used to study the animals. Three regions of the lung were studied by morphologic examination and morphometric analysis: (1) terminal bronchioles -- the terminal sections of the conducting airway that lead directly into respiratory bronchioles and alveolar ducts; (2) the proximal alveolar region -- the alveolar tissue immediately adjacent to the respiratory bronchiole; (3) the distal alveolar region.

Morphometric study of distal alveolar region

Tissue samples were taken from 4 peripheral sites in the left lung of each animal. They are embedded, thin section, and stained for electron microscopic studies. A total of 60 random micrographs were taken from each animal and were printed at a final magnification of 8500X on 11" x 14" photographic paper.

Procedures for carrying out the morphometric analyses of peripheral lung tissue have been well described by Weibel (1979). The proportion of the whole lung volume that is the parenchymal alveolar region was determined by using a light microscope and serial step-cut paraffin sections through the entire right cardiac lobe of each animal. Approximately 1000 uniformly distributed points on the right cardiac lobe were sampled for each animal studied. The percentages falling on the alveolar and non-alveolar regions were determined. Methods for determining the volume, surface, and unmerical densities of tissues and harmonic and arithmetic mean thicknesses were those described by Weibel (1979). Determination of the numerical density of lung cells was done as previously described (Crapo et al., 1978). This requires determining the number of nuclear profiles occurring per unit area of tissue section (these counts were done using ultrathin sections and an electron microscope) and determining the mean caliper diameter D of the major types of lung cell nuclei. The method for determining the mean caliper diameter has been reported previously (Woody et al., 1979).

Morphometric study of the proximal alveolar region

Procedures used to isolate proximal alveolar region were the same as those described by Barry and Crapo (1984). The left lobe of the lung was transversely cut into a series of slices which were examined with a dissecting microscope. Circular cross sections of terminal bronchiles were identified and then punched out with a sharpened 20 gauge Becton Dickenson luer stub cannula. An angled cut was made with a razor blade at the end of each tissue cylinder opposite to that containing the terminal bronchiole. This step facilitated correct orientation of the tissue cylinders in the final stage of embedment. Tissue was prepared for electron microscopy by previously described methods (Crapo, et al., 1980). Using an ultramicrotome, the blocks were serially sectioned through the terminal bronchiole until the first alveolar duct bifurcation was reached. For this study this area and the surrounding alveoli were defined as the proximal alveolar region. Excess tissue was trimmed away and thin sections containing the proximal alveolar region were picked up on too mesh grids. These sections usually covered 10-15 grid squares. Electron micrographs were taken in each of the four corners of

all the grid squares and enlarged to 8500x on 11" x 14" photographic paper. Multipurpose test overlay was placed over each micrograph; the point and intercept counts were tallied to determine volume and surface densities. The number of nuclear profiles of each alveolar cell type in the thin sections was counted directly on the electron microscope to determine the number of cells per unit area, N_A. Numerical densities, N_V, were calculated by dividing N_A values by the mean caliper diameter (D) values, for the alveolar cells of adult male Fischer 344 rats (Pinkerton et al., 1982). It was assumed that inhalation of low concentrations of ozone did not cause changes in the D values. This assumption was based upon the results of earlier studies which found that severe lung injury by oxygen was necessary before significant changes in cell nuclei D occured (Crapo et al., 1980), and that 14 months of inhalation of chrysotile asbestos did not cause any significant changes in these values (Pinkerton et al., 1984).

In contrast to morphometric studies of the proximal alveolar region, values for volume, surface and numerical densities of various tissue components cannot be converted to absolute volumes, surfaces and numbers since the absolute volume of the proximal alveolar region in a rat lung cannot be accurately determined. To report these data as densities alone may cause incorrect interpretation of the data because these values are affected by the degree of lung inflation during fixation. Therefore, the data were normalized by dividing each morphometric parameter by the surface density of epithelial basement membrane in the proximal alveolar region (Barry et al., 1982). Bachofen and Weibel (1982) previously used this technique to express morphometric data from human lungs after acute lung injury. This method reduces variance caused by differences in the degree of inflation of the fixed lungs since the density of tissue and epithelial basement membrane surface area increase or decrease porportionately with different inflation volumes. The rationale for this normalization method is that the basement membrane is a collagenous structure which is thought to undergo minimal stretching at physiological levels of inflation and can, therefore, serve as a stable reference point. When normal rat lungs are fixed by intratracheal instillation of glutaraldehyde at 20 cm of pressure the total lung volume is approximately 70% of total lung capacity as defined by physiologic measurements (Hayatdavoudi, et al., 1980).

Morphometric study of the terminal bronchioles

The method for isolating the terminal bronchiole was esentially the same as described for the proximal alveolar region. Cylinders of tissue thought to contain terminal bronchioles were punched out and embedded. During the initial stages of sectioning, the angle of the knife edge and the tissue blocks were adjusted to correct for misalighment. Sectioning continued until cross sections of terminal bronchioles were obtained. Only terminal bronchioles less than 250 μm in diameter were selected since airways larger than this usually branched and became smaller airways rather than branching into alveolar tissue. Thin sections of 5 terminal bronchioles from each of 6 control and 6 exposed animals were picked up on 1 mm hole parlodion-coated grids. Twenty to twenty-five sequential electron micrographs of the entire terminal bronchiolar epithelium were taken at 2000X and printed at 8500X on 11"x14" photographic paper. Two lines perpendicular to the basement membrane were drawn on each micrograph to avoid counting overlapping areas of

epithelium.

A Merz curvilinear counting overlay was used for this study because of the anisotropy, or preferred orientation, of the terminal bronchiole (Merz 1967). The Merz overlay for this study was composed of semicircles 2 cm in diameter and 224 short hatched lines. This type of overlay minimizes the bias that would be introduced into the intercept counts if an oriented overlay were used on an oriented structure. Point counts are not affected by anisotropy. For volume density calculations, the point immediately adjacent to the right of the intersections of the hatched lines was counted. Points were categorized into different epithelial cell types according to the criteria drawn from previous morphologic studies (Lum et al., 1978; Jeffery and Reid, 1975; Breeze and Wheeldon, 1977; Evans et al., 1978; Jeffery and Reid, 1977; Kuhn et al., 1974; Meyrick and Reid, 1968; Plopper et al., 1980). Intercepts with the basement membrane and luminal surface of each cell type were also tallied. The ratio of the point and intercept counts for each category to the total counts is proportional to each cell type's distribution in the terminal bronchiolar epithelium. The actual tissue area, basement membrane and luminal surface lengths were measured on each micrograph using a Hewlett-Packard 9864 digitizing table interfaced to a Hewlett-Packard 9845A microcomputer. The results were summed to yield total tissue area and basement membrane and luminal surface lengths for each terminal bronchiole. These values were multipled by the point and intercept ratios to calculate volumes and surface densities for each cell category.

The number of nuclear profiles of each cell type per area of epithelium, N_A, were counted on each micrograph and the totals tallied. These values were divided by the mean caliper length of the epithelial cell nuclei, H, to calculate numerical density, N_V. The mean caliper length is the average length of each cell type's nuclei parallel to the direction of sectioning. These values were used rather than D because the terminal bronchioles were cut in a selected orientation. The probability that a nuclear profile is present in a perpendicular cross section of terminal bronchiole is related to the average projected length of the nuclei along the central axis of the terminal bronchiole. D is an inappropriate value to use for the numerical density calculation in this case since it is the mean diameter of the nuclei averaged over all orientations.

The method for determining H is described in the following paragraph. In brief, 60 serial 0.5 μm sections were cut from 2 terminal bronchioles selected from each of 3 control animals (6 total). A segment of epithelium was randomly selected and light micrographs of that segment were taken for the entire series of sections and enlarged to 4500X. The length of nuclei from 7 ciliated and 7 non-ciliated epithelial cells from each terminal bronchiole was determined by counting the number of sections in which a profile of each nucleus was multiplied by 0.5 μm to calculate an individual nuclei caliper length, h. The means of the caliper lengths for each animal were averaged to derive H for each cell type. Since the simple cuboidal terminal bronchiolar epithelium has essentially no basal cell population to serve as a stem cell pool, the basal and unknown cells are actually a mixture of ciliated and non-ciliated epithelial cells but they had too few cytoplasmic characteristics for positive identification. An average of the H's for ciliated and non-ciliated epithelial cells was used for basal and unknown cells.

It was important to know whether ozone exposure caused changes in nuclear size or shape which could affect H. Such changes would invalidate use of the same H values for control and exposed animals. To answer this question a student's t-test was performed on nuclear profile areas from 150 randomly selected non-ciliated epithelial and ciliated cells from control and ozone exposed animals. In addition, histograms of the distribution of the nuclear profile areas were drawn and the degree of skewness and kurtosis determined. No significant differences were found for either cell type. These analyses showed that ozone exposure did not affect the distribution of nuclear profile areas for ciliated and non-ciliated epithelial cells. If exposure had caused changes in nuclear size and shape, these changes should be reflected in the means of the nuclear profile areas and/or in the shape of the histograms of these values. The results indicated that the same H values could be used for control and ozone exposed animals.

The number of cells per mm^2 of basement membrane and their average volumes and surface areas were calculated to demonstrate the distribution and characteristics of cells present in a representative area of terminal bronchiolar epithelium. Since the terminal bronchioles were cut in cross section, the digitized basement membrane length is the circumference of a circle. With circumference known, the surface area of 1 mm of terminal bronchiole can be calculated from the formula: Surface Area = $\pi D1$, where πD = circumference of a circle and 1 is the length of the cylinder. Although no single terminal bronchiole in rat lungs is actually 1 mm long, this value was selected as a convenient unit to express the data. The total tissue volume (V_{Tiss}) in 1 mm of terminal bronchiole is derived by multiplying the digitized epithelial area (A_{Tiss}) by the length. The basement membrane (S_{BM}) and luminal (S_{LM}) surface areas are similarly derived by multiplying their digitized lengths, L_{BM} and L_{LM}, respectively, by 1 mm. Tissue volumes and surface areas for different cell types can be calculated using the volume and surface ratios derived from the point and intercept counts. Mean cell values are calculated by dividing the volume and surface data per mm^2 by the number of each cell type per mm^2 of basement membrane. The surface area contributed by cilia was not directly measured, but instead calculated as a percent increase over the smoothed ciliated cell surface.

Statistics

All comparisons of statistical significance are comparisons of control to treated animals using a two-tailed Student's t-test; $p<0.05$ was considered to be statistically significant.

RESULTS

Effects of Inhalation of 0.25 ppm O_3 on the Distal Alveolar Region of Juvenile and Young Adult Rats

Morphometric methods were used to evaluate the effects of 0.25 ppm ozone on lung tissue randomly selected from the alveolar region of mature and neonatal rats. For this study this area is defined as the distal alveolar region and it includes all alveoli beyond the terminal bronchioles. The results indicate that few structural changes occur in the distal alveolar region as a consequence of this level of O_3 exposure. There were reductions in the body weight gained by mature animals exposed to ozone for 6 weeks ($P=0.05$) and by neonates exposed for 1 week ($P=0.06$). Previous investigators have reported this difference in rats exposed to 1 ppm or less of ozone. The arithmetic mean thicknesses of the epithelium and endothelium increased 10% ($P=0.08$) and 16% ($P=0.08$), respectively, in mature rats. The volume density of air in the alveolar region was significantly smaller in neonatal rats exposed to ozone for 1 week, but this change may have been caused by variations in fixed lung volumes of these small rat lungs. In neonatal rats exposed to ozone for 6 weeks, the average basement membrane surface area covered by type 1 cells decreased 20% ($P=0.06$). There were no significant increases in the arithmetic mean thickness of epithelium or endothelium. No other significant changes were found in the morphometric characteristics of the distal alveolar region of the adult or neonatal rats. Results for this part of the study are presented in Tables 1 through 6.

Effects of Inhalation of 0.25 ppm O_3 on the Proximal Alveolar Region of Juvenile and Young Adult Rats

Since the alveolar region most adjacent to terminal bronchiles has been reported as one of the major sites of injury due to ozone inhalation, this specific lung region was studied using ultrastructural morphometric analysis. After 6 weeks of exposure to 0.25 ppm O_3, qualitative examination of the tissue revealed no observable damage to the terminal bronchioles or the adjacent proximal alveolar tissues. However, by morphometric analysis, significant changes occurred in the alveolar epithelium of the proximal alveolar region. In the animals exposed from 1 day of age (juvenile animals) the number of type I epithelial cells doubled, their mean surface area decreased 38% and their mean thickness increased 24%. The number of alveolar type II epithelial cells increased and the number of alveolar macrophages doubled. Young adult animals exposed to ozone showed similar changes in the epithelium of the proximal alveolar region. The changes in type I and type II cells are illustrated in figures 1 and 2 respectively. Compared to the juvenile animals, the young adult, ozone exposed animals showed more interstitial cell reaction with a doubling of interstitial macrophages suggesting a mild inflammatory stimulus in the interstitium. The change in number and size of type I cells is consistent with an increased cell turnover rate due to prolonged ozone inhalation. These results suggest that 0.25 ppm ozone causes a chronic epithelial injury in the proximal alveolar region of both juvenile and young adult rats. The results of this portion of the study are discussed in more detail in Appendix E.

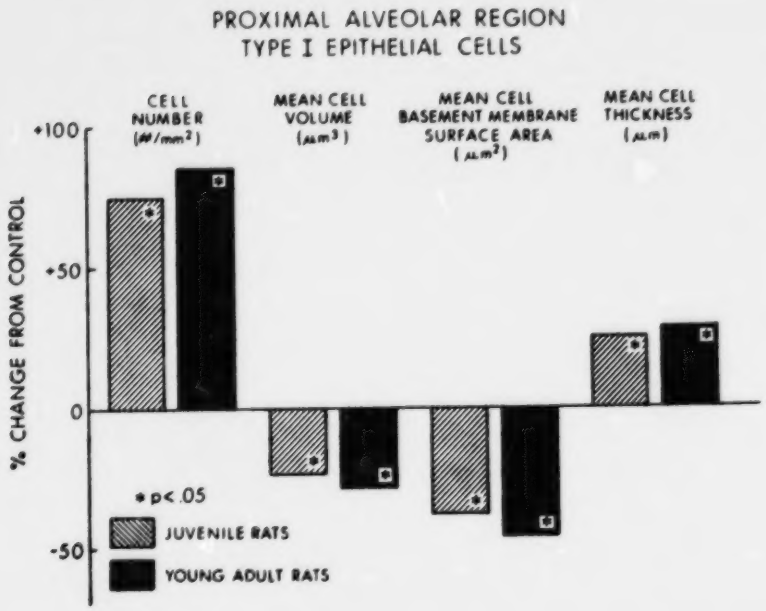

Figure 1. Effects of inhalation of 0.25 ppm ozone on the characteristics of type I epithelial cells in the proximal alveolar region of juvenile and young adult rats.

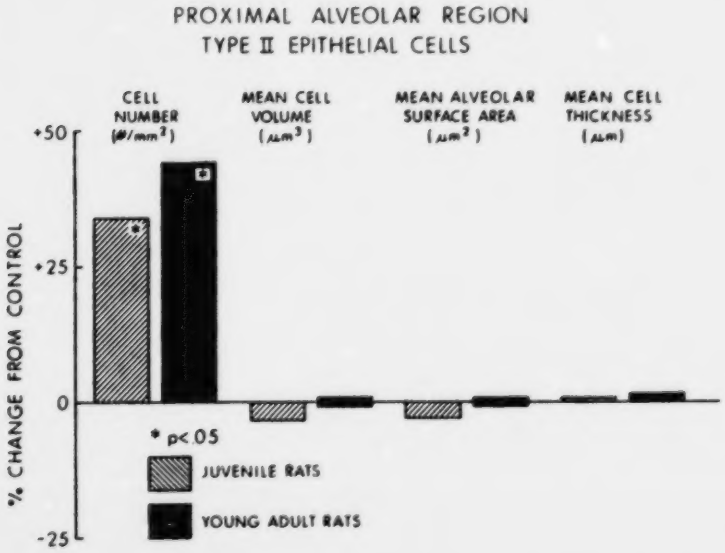

Figure 2. Effects of inhalation of 0.25 ppm ozone on the characteristics of type II epithelial cells in the proximal alveolar region of juvenile and young adult rats.

Effects of Inhalation of 0.12 ppm O_3 on the Proximal Alveolar Region of Young Adult Rats

Morphometric analysis was carried out on lung tissue of the proximal alveolar region isolated from young adult rats that were exposed to 0.12 ppm, the current air quality standard for ozone. This investigation is therefore of fundamental importance in evaluating the efectiveness of present air quality control standards. The results of morphometric analysis indicated that after 6 weeks of ozone inhalation, significant changes occurred in the type I epithelium of the proximal alveolar region. Type I cell number increased 36% ($p < 0.05$) and both their mean surface area and mean basement membrane surface area decreased 23% ($p < 0.05$). While the average type I cell volume decreased 11%, this change was not statistically significant. The volume of type I epithelium increased 13% and the thickness of type II epithelium increased 29%. No significant changes were found in the mean cell volume or surface area of type II cells. The changes observed with the inhalation of 0.12 ppm ozone were found to be similar to, but of lesser magnitude than those occuring with exposure to 0.25 ppm ozone.

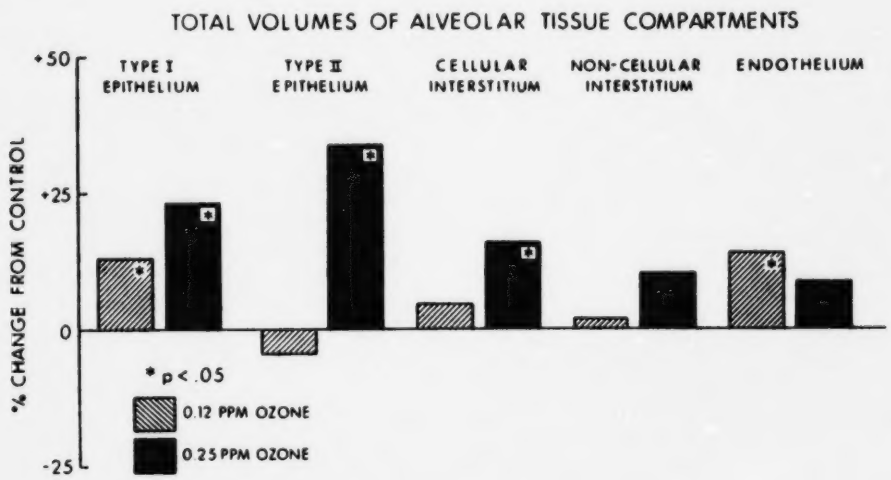

Figure 3. Effects of inhalation of 0.12 ppm or 0.25 ppm ozone on the total tissue volumes of the various tissue compartments in the proximal alveolar region of young adult rats.

The higher concentration of ozone produced both a greater and a broader degree of injury. In addition to increases in type I epithelium, the volumes of type II epithelium, alveolar macrophage and cellular interstitium were found to increase after 6 weeks of exposure to 0.25 ppm ozone. This study demonstrates that the exposures to ozone concentrations near current air quality standard

of 0.12 ppm ozone can cause measurable structural changes in the alveolar epithelium. The degree of tissue injury caused by ozone can be rigerously quantified and increased with exposure to higher concentrations of ozone.

Results of morphometric analysis of tissue volume and cell numbers change after exposure to 0.12 ppm O_3 are presented in Tables 7 and 8.

Effects of Inhalation of 0.25 ppm O_3 on the Terminal Bronchioles of Juvenile and Young Adult Rats

Terminal bronchioles comprise the final portion of the conducting airway in the lung. It has been shown to be particularly sensitive to the effects of ozone exposure. Morphometric methods were developed to evaluate the effects of inhalation of 0.25 ppm ozone on the terminal bronchioles of rats. This concentration of ozone is near the current air quality standard of 0.12 ppm and is below the level at which changes can be reliably documented qualitatively. The results of the morphometric analysis indicated that ciliated cells composed 48% (juvenile) or 53% (young adult) of the total population of terminal bronchiolar cells and clara cells were 33% (juvenile) or 29% (young adult). The average terminal bronchiolar diameter (210 µm) and the average thickness of the bronchiolar epithelium (7.5 µm) was not significantly changed by the exposure to ozone. Exposure to 0.25 ppm ozone for 6 weeks produced a significant 14-16% decrease ($p < 0.05$) in the average luminal surface area of clara cells in the terminal bronchioles of both juvenile and young adult rats. No changes occurred in mean clara cell volume or in average clara cell basement membrane surface area. The changes found in ciliated cells are illustrated below.

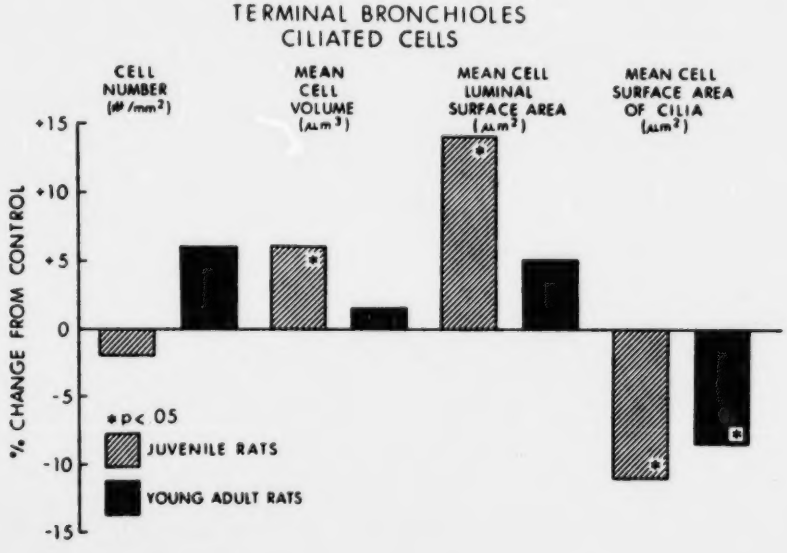

Figure 4. Effects of inhalation of 0.25 ppm ozone on the characteristics of ciliated cells in the terminal bronchioles of juvenile and young adult rats.

The effect of ozone on terminal bronchiolar cells are subtle, especially when compared with changes induced in the epithelium of the proximal alveolar region after exposure to the same concentrations of ozone. These differences may be due to the terminal bronchiolar epithelial cells being less susceptible to this concentration of ozone than are the cells of the alveolar epithelium. Alternatively, it may be due to the fact that the epithelium in the terminal bronchiole is covered by a mucous/serous coat which may react with the low concentrations of O_3 and thereby prevent the O_3 from reaching the underlying cells.

Tables 9 and 10 list the characteristics of terminal bronchiolar cells in control and exposed animals of both juvenile and young adult rats.

DISCUSSION

The use of morphometric techniques to evaluate patterns of tissue and cell reactions after exposure to oxidants permits a more rigorous comparison of both the extent and distribution of effects caused by different agents.

The site of injury for a gas with relatively low toxicity but present in high concentration such as oxygen depends on the susceptibility of the individual cell population since it is uniformly distributed throughout the alveolar region and rapidly diffuses across tissues (Barry and Crapo, 1984).

Ozone, on the other hand, represents an oxidant for which the pattern of lung injury is likely to be highly influenced by the distribution of the gas. Ozone has two unpaired electrons in its outer shell which give it the potential to oxidize many cell components (Mustafa, et al., 1978), possibly through free radical formation (Menzel, 1976). Lipids in plasma membranes can undergo peroxidation or ozonolysis after exposure to ozone. Similar reactions with proteins and amino acids can lead to altered enzymatic activities and toxic effects on the cell (Mudd and Freeman, 1977). All of the above mechanisms suggest that ozone may react rapidly with tissues contacted in crossing the alveolar septum. The concentration of the lesions caused by ozone in the proximal portions of the acinus may be due to the fact that the mass movement of fresh gas during each inspiration ends in the proximal acinus and movement of the gas into the distal alveoli is dependent on diffusion (Farhi, 1969). The reaction of the oxidants with tissue components in the proximal portions of the acinus is presumed to rapidly reduce the concentrations of the oxidants as they flow toward distal alveoli. Thus, the toxic response would tend to occur most intensely in the distal airways and proximal portions of the acinus and would also tend to involve tissues closest to the gas-tissue interface.

In evaluating the reaction to oxidants present in low concentrations morphometry offers a sensitive, quantitative tool to detect changes which may be difficult to substantiate by qualitative means alone. Previous investigators (Plopper, et al., 1979; Schwartz, et al., 1976) have reported slight changes in terminal bronchioles and in the adjacent alveoli in rats after exposure to 0.2 ppm ozone, but no changes in the alveoli beyond this region were found. Our data support the concept that low doses of ozone will not cause injury to the distal portions of the alveolar region of the lung but that injury does occur in the proximal alveolar region. In this region the numbers of type I and type II cells change in patterns that are consistent with injury to the alveolar epithelium causing loss of alveolar type I epithelial cells and proliferation of alveolar type II epithelial cells in order to regenerate and replace damaged type I epithelium. Type I cells were smaller in volume, covered less surface area and were thicker than those of control animals. Differentiation of a type II cell into a type I cell requires more than a four fold increase in mean cell volume and a 48 fold increase in average surface area. The changes found to occur in type I epithelium after exposure to low doses of O_3 suggest that a portion of these cells are at an intermediate stage of cell differentiation between type I and type II cells.

Both the qualitative evidence of epithelial cell hypertrophy and injury and the morphometric changes in the characteristics of type I and type II epithelial cells (Figures 1 and 2) suggest that inhalation of 0.12 or 0.25 ppm ozone will cause type I epithelial cell injury and an increase in the rate of epithelial cell proliferation. This conclusion is supported by the work of Evans, et al., (1976) who found increased mitotic activity in alveolar type II epithelial cells in the proximal alveolar region of rats exposed to less than 1 ppm of ozone. Castleman, et al., (1973a, 1973b) used histochemical methods to demonstrate an increase in the amount of acid phosphatase and β-glucuronidase present in epithelial cells of terminal bronchiles and their adjacent alveoli after exposure of rats to ozone. They postulated that ozone may have damaged the plasma membrane of lysosomes and allowed leakage of lysosomal enzymes into the cell cytoplasm. Thus, the results of morphometric, autoradiographic and histochemical studies all suggest low doses of O_3 can cause injury to type I epithelial cells and increase rates of epithelial cell turnover in the proximal alveolar region of the lung.

Other changes noted by morphometry were that macrophages increased by 50 to 100% and there was a small increase in the volume of the cellular components of the interstitium. Hussain, et al., (1976) have demonstrated an increased concentration of prolyl hydroxylase and hydroxyproline in rat lung homogenates after exposure of animals to 0.8 ppm ozone for 7 days. We qualitatively identified evidence of increased collagen in the proximal alveolar region. Histologic evidence of fibrotic lesions caused by higher doses of ozone has been reported (Freeman, et al., 1974) and ozone has been used to produce pulmonary fibrosis in rats (Hesterberg and Last, 1981). The small changes in the volume of the interstitium are likely to be associated with a mild but significant chronic fibrotic reaction.

Evidence of significant injury to the capillary endothelium after exposure to ozone was not found. The number of endothelium cells, their average volume and average surface area did not change other than there being a small increase in average volume of endothelial cells after exposure to 0.25 ppm ozone. The most likely reason for the difference in degree of injury occurring in alveolar epithelial cells and endothelial cells is that the oxidant gas reacts with tissue components as it diffuses across the alveolar septum. This would give a relatively large dose to the epithelium while reducing the dose to which the endothelial cells are exposed.

An important question addressed in this study was whether there were differences in the effects of ozone inhalation between mature and developing rat lungs. The mature rats represented an adult lung model while neonatal rats, which literally grew up inhaling ozone, represented a growing lung. Several differences were found between young adult and juvenile rats. In the distal alveolar region, increases in the arithmetic mean thickness of the epithelium and endothelium were found with the young adult but not neonatal rats. In the proximal alveolar region, ozone inhalation caused significant increases in the total volumes of interstitial and endothelial cells of adult rats which were not found in the neonates. These differences between adults and neonates is probably related to age. Stephens et al., (1978, 1982) have reported that postnatal rats are resistant to the toxic effects of ozone and

nitrogen dioxide until they are weaned. After this point there is progressive susceptibility to the effects of these gases in the proximal alveolar region. Endogenous antioxidant systems in rats prior to weaning (Yam et al., 1978) or the effects of change in diet from mother's milk to rat chow are potential reasons for this resistance. Since the neonate group in the present study was exposed from 1 day to 6 weeks of age, the morphometric and morphologic changes which were found may have primarily occurred after the animals were weaned at 3 weeks of age. The more extensive changes in the adult rats may be due to lack of similar protection during their entire 6 week exposure to ozone.

The terminal bronchiole and proximal alveolar regions are of significant functional importance in the lung since this is the critical intersection between the conductive and respiratory portions of the lung. This area is a focal point of several pathologic lesions, including centrilobular emphysema, asbestos fiber deposition (Brody etal., 1982) and injury caused by oxidant gases such as ozone and nitrogen dioxide. Loss of alveolar tissue, as seen in centrilobular emphysema, or fibrotic changes, which are caused by inhalation of particulates and oxidant gases, can affect normal gas exchange in this region. This may alter the distribution of air to more distal portions of the lung parenchyma. The present study demonstrated that inhalation of 0.12 ppm O_3 for 6 weeks caused structural alteration in the proximal alveolar region. The effect of chronic exposure to this level of O_3 in an exposure pattern relevant to the urban environment should, therefore, be closely examined in order to determine the progression (or the lack of progression) of chronic lung disease in animals exposed chronically to low levels of ozone.

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TABLE 1

ABSOLUTE VOLUMES, SURFACE AREAS AND DIFFUSION CAPACITY FOR PARENCHYMAL PORTION OF THE LUNGS OF 12 WEEK OLD RATS EXPOSED TO 0.25 PPM OZONE SINCE 6 WEEKS OF AGE
DISTAL ALVEOLAR REGION

	Controls	0.25 PPM Ozone
n	8	8
Body Weight (gm)	268 ± 4	249 ± 8*
Lung Volume (ml)	7.85 ± 0.17	7.97 ± 0.26
Total Volumes (cm^3/both lungs)		
Air	5.600 ± 0.133	5.179 ± 0.214
Capillary Lumen	0.439 ± 0.038	0.410 ± 0.038
Tissue	0.322 ± 0.014	0.322 ± 0.024
Type I Epithelium	0.071 ± 0.005	0.075 ± 0.006
Type II Epithelium	0.023 ± 0.002	0.021 ± 0.004
Cellular Interstitium	0.061 ± 0.005	0.053 ± 0.005
Non-cellular Interstitium	0.072 ± 0.006	0.071 ± 0.007
Endothelium	0.087 ± 0.005	0.094 ± 0.007
Macrophages	0.008 ± 0.002	0.007 ± 0.002
Surface Area (cm^2/both lungs)**		
Alveolar Epithelium	3165 ± 160	2967 ± 181
Type I	3064 ± 170	2851 ± 171
Type II	101 ± 17	117 ± 27
Capillary Endothelium	3376 ± 119	3154 ± 270
D_T (ml $O_2 \cdot min^{-1} \cdot mm\ Hg^{-1}$)	2.969 ± 0.178	2.604 ± .173
Tissue Thickness (μm)		
Harmonic Mean (Air to Plasma)	0.367 ± 0.014	0.388 ± 0.007
Arithmetic Mean		
Epithelium	0.298 ± 0.011	0.327 ± 0.020
Type I	0.231 ± 0.010	0.267 ± 0.017*
Type II	2.569 ± 0.351	2.185 ± 0.449
Interstitium	0.409 ± 0.018	0.412 ± 0.037
Endothelium	0.261 ± 0.014	0.303 ± 0.017*

All data are mean ± S.E.

*$p < 0.10$

**Type I SA is the SA of basement membrane under Type I cells; Type II SA is the air surface of Type II cells excluding the extra SA contributed by microvilli; endothelial SA is the luminal surface of the endothelial cells.

TABLE 2

MORPHOMETRICALLY DETERMINED CHARACTERISTICS OF THE MAJOR PARENCHYMAL LUNG CELLS IN 12 WEEK OLD RATS EXPOSED TO 0.25 PPM OZONE SINCE 6 WEEKS OF AGE DISTAL ALVEOLAR REGION

	Control	0.025 ppm Ozone
n	8	8
Total Number of Cells Per Both Lungs ($\times 10^6$)	533 ± 28	520 ± 17
Alveolar Type I Cells		
Total Number ($\times 10^6$)	39 ± 3	42 ± 3
Average Volume (μm^3)	1860 ± 138	1920 ± 237
Average Surface Area (μm^2)	9412 ± 849	8469 ± 958
Average Basement Membrane SA/Cell (μm^2)	8189 ± 720	7203 ± 795
% Total Lung Cells	7	8
% Alveolar Surface Covered	97	96
Alveolar Type II Cells		
Total Number ($\times 10^6$)	59 ± 3	60 ± 3
Average Volume (μm^3)	401 ± 47	340 ± 59
Average Surface Area (μm^2)		
with microvilli	178 ± 39	219 ± 47
without microvilli	177 ± 32	195 ± 42
% Total Lung Cells	11	12
% Alveolar Surface Covered	3	4
Endothelial Cells		
Total Number ($\times 10^6$)	296 ± 14	294 ± 11
Average Volume (μm^3)	298 ± 18	327 ± 31
Average Surface Area (μm^2)		
% Total Lung Cells	55	57
Interstitial Cells		
Total Number ($\times 10^6$)	131 ± 9	116 ± 6
Average Volume (μ^3)	484 ± 56	475 ± 56
% Total Lung Cells	25	22
Macrophages		
Total Number ($\times 10^6$)	9 ± 1	9 ± 1
Average Volume (μm^3)	1044 ± 396	959 ± 299
% Total Lung Cells	2	2

All data are mean ± S.E.

*p 0.10.

TABLE 3

ABSOLUTE VOLUMES, SURFACE AREAS AND DIFFUSION CAPACITY FOR PARENCHYMAL PORTION OF THE LUNGS OF 1 WEEK OLD RATS EXPOSED TO 0.25 PPM OZONE SINCE 1 DAY OF AGE
DISTAL ALVEOLAR REGION

	Controls	0.25 PPM Ozone
n	8	8
Body Weight (gm)	11.39 ± .46	10.14 ± .43*
Lung Volume (ml)	.993 ± .028	.884 ± .052*
Total Volumes (cm^3/both lungs)		
Air	.680 ± .018	.594 ± .031*
Capillary Lumen	.023 ± .002	.022 ± .004
Tissue	.101 ± .010	.100 ± .009
Type I Epithelium	.009 ± .001	.010 ± .001
Type II Epithelium	.005 ± .001	.005 ± .001
Cellular Interstitium	.053 ± .005	.051 ± .006
Non-cellular Interstitium	.012 ± .001	.014 ± .001
Endothelium	.020 ± .002	.019 ± .002
Macrophages	.001 ± .000	.001 ± .000
Surface Area (cm^2/both lungs)		
Alveolar Epithelium	308 ± 21	311 ± 33
Type I	287 ± 18	290 ± 30
Type II	20 ± 5	21 ± 5
Capillary Endothelium	298 ± 18	294 ± 41
D_T (ml $O_2 \cdot min^{-1} \cdot mm\ Hg^{-1}$)	.189 ± .022	.176 ± .03
Tissue Thickness (μ)		
Harmonic Mean (Air to Plasma)	.555 ± .037	.615 ± .054
Arithmetic Mean		
Epithelium	.472 ± .032	.491 ± .035
Type I	.327 ± .022	.347 ± .029
Type II	.612 ± .738	.778 ± .312
Interstitium	.127 ± .131	.178 ± .073
Endothelium	.660 ± .52	.686 ± .047

All data are mean ± S.E.

*p <0.10
**Type I SA is the SA of basement membrane under Type I cells; Type II SA is the air surface of Type II cells excluding the extra SA contributed by microvilli; endothelial SA is the luminal surface of the endothelial cells.

TABLE 4

MORPHOMETRICALLY DETERMINED CHARACTERISTICS OF THE MAJOR PARENCHYMAL LUNG CELLS IN 1 WEEK OLD RATS EXPOSED TO 0.25 PPM OZONE SINCE 1 DAY OF AGE
DISTAL ALVEOLAR REGION

	Control	0.025 ppm Ozone
n	8	8
Total Number of Cells Per Both Lungs ($x10^6$)	203 ± 16	185 ± 10
Alveolar Type I Cells		
Total Number ($x10^6$)	14 ± 1	13 ± 1
Average Volume (μm^3)	701 ± 57	757 ± 52
Average Surface Area (μm^2)	2317 ± 98	2495 ± 243
Average Basement Membrane SA/Cell (μm^2)	2133 ± 76	2292 ± 230
% Total Lung Cells	7	7
% Alveolar Surface Covered	93	93
Alveolar Type II Cells		
Total Number ($x10^6$)	16 ± 1	14 ± 1
Average Volume (μm^3)	315 ± 37	360 ± 33
Average Surface Area (μm^2)		
with microvilli	132 ± 25	155 ± 13
without microvilli	122 ± 27	149 ± 32
% Total Lung Cells	8	8
% Alveolar Surface Covered	7	7
Endothelial Cells		
Total Number ($x10^6$)	64 ± 6	55 ± 4
Average Volume (μm^3)	320 ± 31	352 ± 20
Average Surface Area (μm^2)	498 ± 59	525 ± 41
% Total Lung Cells	32	30
Interstitial Cells		
Total Number ($x10^6$)	108 ± 9	102 ± 6
Average Volume (μm^3)	490 ± 25	495 ± 33
% Total Lung Cells	53	55
Macrophages		
Total Number ($x10^6$)	1.4 ± 0.2	1.5 ± 0.3
Average Volume (μm^3)	766 ± 116	498 ± 142
% Total Lung Cells	1	1

* $p<0.10$
All data are mean ± S.E.

TABLE 5

ABSOLUTE VOLUMES, SURFACE AREAS AND DIFFUSION CAPACITY FOR PARENCHYMAL PORTION OF THE LUNGS OF 6 WEEK OLD RATS EXPOSED TO 0.25 PPM OZONE SINCE 1 DAY OF AGE
DISTAL ALVEOLAR REGION

	Controls	0.25 PPM Ozone
n	8	8
Body Weight (gm)	130 ± 5	129 ± 2
Lung Volume (ml)	5.39 ± 0.17	5.40 ± 0.14
Total Volumes (cm^3/both lungs)		
Air	3.880 ± 0.110	3.890 ± 0.094
Capillary Lumen	0.224 ± 0.018	0.221 ± 0.017
Tissue	0.263 ± 0.017	0.258 ± 0.021
Type I Epithelium	0.050 ± 0.004	0.051 ± 0.005
Type II Epithelium	0.018 ± 0.003	0.018 ± 0.004
Cellular Interstitium	0.070 ± 0.006	0.070 ± 0.008
Non-cellular Interstitium	0.047 ± 0.004	0.046 ± 0.004
Endothelium	0.072 ± 0.006	0.066 ± 0.005
Macrophages	0.006 ± 0.001	0.008 ± 0.002
Surface Area (cm^2/both lungs)**		
Alveolar Epithelium	2109 ± 89	1903 ± 118
Type I	2029 ± 86	1812 ± 112
Type II	80 ± 17	91 ± 29
Capillary Endothelium	2154 ± 160	2068 ± 120
D_T (ml $O_2 \cdot min^{-1} \cdot mm\ Hg^{-1}$)	1.758 ± 0.144	1.634 ± 0.131
Tissue Thickness (μ)		
Harmonic Mean (Air to Plasma)	0.407 ± 0.013	0.412 ± 0.025
Arithmetic Mean		
Epithelium	0.322 ± 0.026	0.357 ± 0.028
Type I	0.249 ± 0.021	0.276 ± 0.016
Type II	2.985 ± 0.593	2.548 ± 0.342
Interstitium	0.548 ± 0.026	0.578 ± 0.050
Endothelium	0.333 ± 0.012	0.319 ± 0.015

All data are mean ± S.E.

* $p<0.10$
**Type I SA is the SA of basement membrane under Type I cells; Type II SA is the air surface of Type II cells excluding the extra SA contributed by microvilli; endothelial SA is the luminal surface of the endothelial cells.

TABLE 6

MORPHOMETRICALLY DETERMINED CHARACTERISTICS OF THE MAJOR PARENCHYMAL LUNG CELLS IN 6 WEEK OLD RATS EXPOSED TO 0.25 PPM OZONE SINCE 1 DAY OF AGE
DISTAL ALVEOLAR REGION

	Control	0.025 ppm Ozone
n	8	8
Total Number of Cells Per Both Lungs ($x10^6$)	436 ± 18	429 ± 18
Alveolar Type I Cells		
Total Number ($x10^6$)	27 ± 2	30 ± 3
Average Volume (μm^3)	1922 ± 155	1692 ± 85
Average Surface Area (μm^2)	8966 ± 615	7061 ± 547*
Average Basement Membrane SA/Cell (μm^2)	7874 ± 574	6310 ± 523*
% Total Lung Cells	6	7
% Alveolar Surface Covered	96	95
Alveolar Type II Cells		
Total Number ($x10^6$)	51 ± 2	50 ± 2
Average Volume (μm^3)	337 ± 41	366 ± 70
Average Surface Area (μm^2)		
with microvilli	243 ± 46	192 ± 44
without microvilli	151 ± 34	186 ± 63
% Total Lung Cells	12	12
% Alveolar Surface Covered	4	5
Endothelial Cells		
Total Number ($x10^6$)	234 ± 12	226 ± 12
Average Volume (μm^3)	307 ± 19	292 ± 15
Average Surface Area (μm^2)	920 ± 46	917 ± 34
% Total Lung Cells	54	53
Interstitial Cells		
Total Number ($x10^6$)	116 ± 5	114 ± 3
Average Volume (μm^3)	605 ± 55	603 ± 63
% Total Lung Cells	26	26
Macrophages		
Total Number ($x10^6$)	8.3 ± 0.5	9.2 ± 1.5
Average Volume (μm^3)	790 ± 205	949 ± 269
% Total Lung Cells	2	2

* $p<0.10$
All data are mean ± S.E.

TABLE 7

ABSOLUTE VOLUMES, SURFACE AREAS AND DIFFUSION CAPACITY FOR PARENCHYMAL PORTION OF THE LUNGS OF 12 WEEK OLD RATS EXPOSED TO 0.12 PPM OZONE SINCE 6 WEEKS OF AGE
PROXIMAL ALVEOLAR REGION

	Controls	0.12 PPM Ozone
n	12	8
Total Lung Volume (cm^3)	8.12±0.22	8.20±0.17
Basement Membrane Surface Density (cm^2/cm^3)	493±14	479±15
Volumes ($V/S_{Ep}BM$) (μm)		
Air	18.156±0.569	18.769±0.581
Capillary Lumen	1.315±0.057	1.193±0.160
Tissue	0.996±0.025	1.075±0.038
Type I Epithelium	0.193±0.005	0.218±0.007*
Type II Epithelium	0.074±0.005	0.071±0.008
Cellular Interstitium	0.195±0.008	0.203±0.012
Non-cellular Interstitium	0.264±0.007	0.268±0.016
Endothelium	0.250±0.007	0.284±0.009*
Macrophages	0.019±0.002	0.032±0.008
Surface Area**		
% Type I	98.1±0.2	98.8±0.2*
% Type II	1.9±0.2	1.2±0.2
Capillary Endothelium	96.6±0.1	98.3±2.3
D_T ($\mu l\ O_2 \cdot min^{-1} \cdot mm\ Hg^{-1} \cdot cm^{-2}$)	0.876±0.024	0.883±0.027
Tissue Thickness (μ)		
Harmonic Mean (Air to Plasma)	0.3728±0.0089	0.3725±0.0091
Arithmetic Mean		
Epithelium	0.267±0.081	0.288±0.008
Type I	0.197±0.005	0.220±0.007*
Type II	4.416±0.372	5.682±0.417*
Interstitium	0.467±0.015	0.474±0.021
Endothelium	0.260±0.008	0.290±0.11*

All data are mean ± S.E.

* $p < 0.05$

** Type I SA is the SA of basement membrane under Type I cells; Type II SA is the air surface of Type II cells excluding the extra SA contributed by microvilli; endothelial SA is the luminal surface of the endothelial cells expressed as a percent relative to the alveolar basement membrane surface area.

TABLE 8

MORPHOMETRICALLY DETERMINED CHARACTERISTICS OF THE MAJOR PARENCHYMAL LUNG CELLS IN 12 WEEK OLD RATS EXPOSED TO 0.12 PPM OZONE SINCE 6 WEEKS OF AGE
PROXIMAL ALVEOLAR REGION

	Control	0.12 PPM Ozone
n	12	8
Total Number of Cells (/mm²)	1611 ± 58	1756 ± 80
Alveolar Type I Cells		
Number (/mm²)	103 ± 6	140 ± 15*
Average Volume (μm³)	1947 ± 144	1730 ± 266
Average Surface Area (μm²)	10297 ± 717	7928 ± 948*
Average Basement Membrane SA/Cell (μm²)	9910 ± 720	7690 ± 834*
% Total Lung Cells	6	8
Alveolar Type II Cells		
Number (/mm²)	171 ± 12	212 ± 26
Average Volume (μm³)	447 ± 33	357 ± 52
Average Surface Area (μm²)		
with microvilli	209 ± 29	154 ± 21
without microvilli	109 ± 12	64 ± 9*
% Total Lung Cells	11	12
Endothelial Cells		
Number (/mm²)	885 ± 24	943 ± 37
Average Volume (μm³)	284 ± 8	303 ± 10
Average Surface Area (μm²)	1101 ± 34	1057 ± 55
% Total Lung Cells	55	54
Interstitial Cells		
Number (/mm²)	426 ± 33	433 ± 18
Average Volume (μm³)	488 ± 40	469 ± 22
% Total Lung Cells	26	25
Macrophages		
Number (/mm²)	25 ± 3	27 ± 5
Average Volume (μm³)	945 ± 197	1209 ± 252
% Total Lung Cells	2	2

All data are mean ± S.E.

* $p < 0.05$

TABLE 9

MORPHOMETRICALLY DETERMINED CHARACTERISTICS OF TERMINAL BRONCHIOLAR CELLS IN THE LUNGS OF 6 WEEK OLD RATS EXPOSED TO 0.25 PPM OF OZONE SINCE 1 DAY OF AGE

	Control	0.25 ppm Ozone
n	8	8
Average Terminal Bronchiole Diameter(μm)	202 ± 3	196 ± 4
Average Epithelial Thickness (μm)	7.32 ± 0.11	7.64 ± 0.14
Total Number of Cells per mm^2 of Basement Membrane	17175 ± 329	16597 ± 363
Ciliated Cells		
Number per mm^2	8183 ± 253	7981 ± 144
Average Volume (μm^3)	419 ± 9	444 ± 5*
Average Basement Membrane Surface Area (μm^2)	71 ± 2	73 ± 2
Average Luminal Surface Area (μm^2)		
Total	293 ± 17	271 ± 13
Without cilia	57 ± 1	65 ± 2*
Cilia only	236 ± 17	206 ± 12
% Total Cells per mm^2	48	48
% Basement Membrane Surface Covered per mm^2	56	54
Non-Ciliated Bronchiolar (Clara) Cells		
Number per mm^2	5661 ± 123	5678 ± 134
Average volume (μm^3)	520 ± 13	546 ± 17
Average Basement Membane Surface Area (μm^2)	45 ± 2	50 ± 2
Average luminal surface area (μm^2)	122 ± 5	92 ± 2*
% Total Cells per mm^2	33	34
% Basement Membrane Surface Covered per mm^2	26	26
Brush Cells		
Number per mm^2	473 ± 60	379 ± 62
Average Volume(μm^3)	366 ± 35	338 ± 20
Average Basement Membrane Surface Area (μm^2)	39 ± 6	36 ± 5
Average Luminal Surface Area (μm^2)	32 ± 5	22 ± 6
% Total Cells per mm^2	3	2
% Basement Membrane Surface Covered per mm^2	1	1
Unknown Cells		
Number per mm^2	2258 ± 195	1934 ± 228
Average Volume (μm^3)	238 ± 15	279 ± 19
Average Basement Membrane Surface Area (μm^2)	75 ± 4	81 ± 5
Average Luminal Surface Area (μm^2)	10 ± 1	17 ± 6
% Total Cells	13	12
% Basement Membrane Surface Covered per mm^2	14	11

All data are mean ± S.E.
* $p < 0.05$

TABLE 10

MORPHOMETRICALLY DETERMINED CHARACTERISTICS OF TERMINAL BRONCHIOLAR CELLS IN THE LUNGS OF 12 WEEK OLD RATS EXPOSED TO 0.25 PPM OF OZONE SINCE 6 WEEKS OF AGE

	Control	0.25 ppm Ozone
n	8	8
Average Terminal Bronchiole Diameter (μm)	204 ± 4	208 ± 5
Average Epithelial Thickness (μm)	7.45 ± 0.28	7.85 ± 0.19
Total Number of Cells per mm² of Basement Membrane	17177 ± 451	17535 ± 606
Ciliated Cells		
Number per mm²	9122 ± 343	9666 ± 345
Average Volume (μm³)	415 ± 10	425 ± 15
Average Basement Membrane Surface Area (μm²)	66 ± 1	62 ± 2
Average Luminal Surface Area (μm²)		
Total	341 ± 6	325 ± 12
Without cilia	56 ± 2	59 ± 2
Cilia only	280 ± 8	267 ± 11
% Total Cells per mm²	53	55
% Basement Membrane Surface Covered per mm²	57	58
Non-Ciliated Bronchiolar (Clara) Cells		
Number per mm²	5016 ± 113	5164 ± 266
Average volume (μm³)	550 ± 13	566 ± 21
Average Basement Membane Surface Area (μm²)	47 ± 2	52 ± 2
Average luminal surface area (μm²)	125 ± 5	105 ± 8*
% Total Cells per mm²	29	29
% Basement Membrane Surface Covered per mm²	23	26
Brush Cells		
Number per mm²	606 ± 51	526 ± 57
Average Volume (μm³)	311 ± 18	284 ± 18
Average Basement Membrane Surface Area (μm²)	43 ± 2	36 ± 4
Average Luminal Surface Area (μm²)	21 ± 3	18 ± 4
% Total Cells per mm²	4	3
% Basement Membrane Surface Covered per mm²	2	1
Unknown Cells		
Number per mm²	2243 ± 211	2018 ± 178
Average Volume (μm³)	223 ± 13	258 ± 31
Average Basement Membrane Surface Area (μm²)	72 ± 3	68 ± 4
Average Luminal Surface Area (μm²)	11 ± 3	10 ± 3
% Total Cells	13	12
% Basement Membrane Surface Covered per mm²	15	12

All data are mean ± S.E.
* $p < 0.05$